EFFECT OF GUANIDINIUM ION ON DNA DENATURATION AND RENATURATION

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Guanidinium chloride (GuCl) in concentrated solutions has been used in denaturing proteins (Tanford, Kawachara and Lapanje 1967) and for this reason, it has also been suggested for use in the isolation of nucleic acids from nucleoproteins (Cox 1968). While there is no apparent effect of this procedure on the products so isolated, little has been reported as to its action on DNA alone. Rice and Doty (1957) observed earlier that the decrease in intrinsic viscosity of calf thymus DNA occurred at a lower temperature in 3.2 M GuCl than in 0.8 M NaCl, but 8 M urea was more effective than 3.2 M GuCl. The present studies employ the measurement of the absorbance-temperature profile (Rice and Doty 1957, Cavalieri and Rosenberg 1957, Marmur and Doty 1962) and show that the degree of stabilization of DNA helicity by GuCl at low ionic strength is proportional to the molarity of the salt, whereas at high concentrations, the reverse is the case. Thus, the results suggest a unique, dual effect of GuCl uncommon to most denaturants.

Results and Discussion

The relationship between GuCl concentration and the temperature corresponding to the midpoint of the absorbance rise, Tm is shown in Fig. 1. The effects of NaCl and urea are also included for comparison. At 1×10^{-3} M to 0.5 M of GuCl, the Tm increases almost linearly with the logarithm of molarity with a slope of about 8.5°C per log. This effect is similar to a number of low molecular weight salts which also stabilize the DNA helical structure (Dove and Davidson 1962, Colvill and Jordan 1963, Kotin 1963). However, the effects persist at high concentrations for these common salts (see for instance the NaCl effect in Fig. 1).

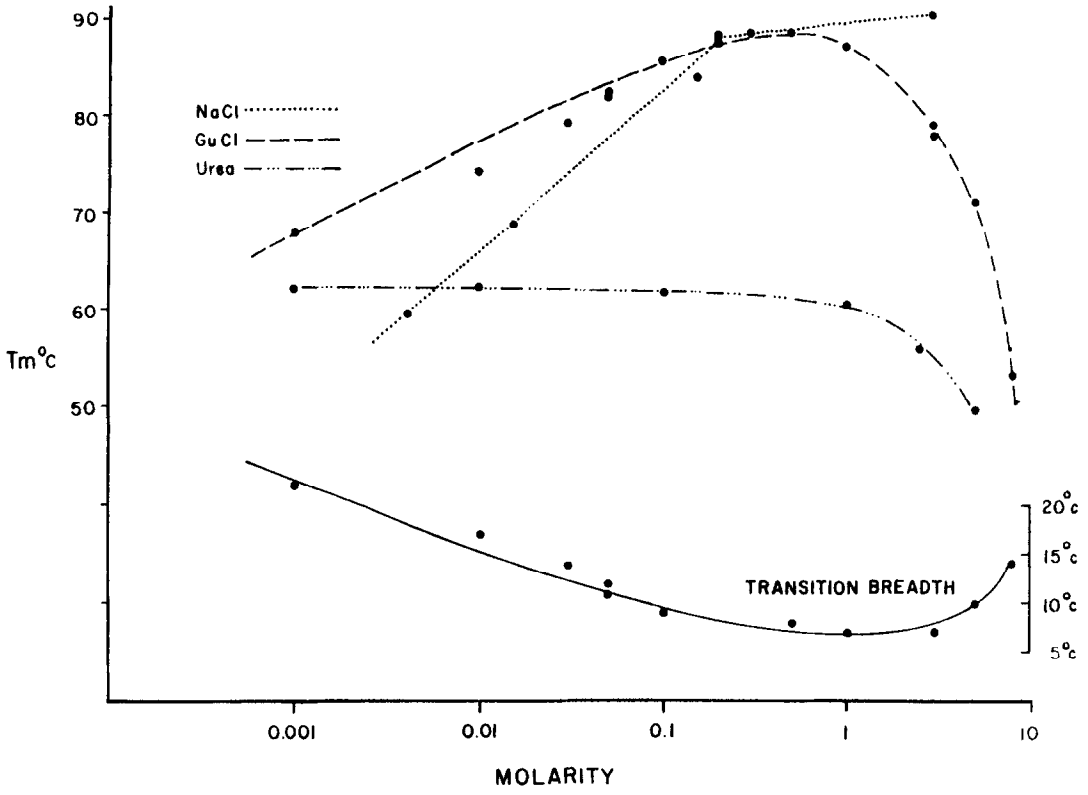

Fig. 1 - Tm and transition breadth of DNA during denaturation as affected by solvent environment. Data obtained by replotting temperature-absorbance profiles.

At high concentrations (>0.5 M) of GuCl, the Tm decreases linearly with the molarity of the salt. It seems that a disruption of the ordered helical structure is involved. This effect is similar to that seen with urea (Alexander and Stacey 1957, Rice and Doty 1957, Herskovits 1963), also a denaturant for proteins and with $C Cl_3 COO^-$ at high concentrations (Hamaguchi and Geiduschek 1962).

The effect of GuCl on the transition breadth is also shown in Fig. 1. Transition breadth is defined here as the temperature range required to bring about a full hyperchromicity (Hamaguchi and Geiduschek 1962). It can be seen from Fig. 1 that the transition breadth is broader (14^o) at both high and low concentrations of GuCl with a minimum (7^o) attained at about 1.0 M. As a

narrower temperature range is required to achieve a complete denaturation, it would appear as though that there is a greater degree of cooperativity for the process under such conditions.

The renaturation of the heat denatured DNA in the presence of GuCl is of considerable interest here. Figure 2 shows some examples of denaturation and renaturation of DNA in higher concentrations of GuCl with or without urea. Not shown in the figure is that at or below 0.01 M, there is very little renaturation under the slow cooling conditions used, indicating that the rate of renaturation (Crothers 1964, Wetmur and Davidson 1968), if any, is very low. Partial renaturation is observed in the range of 0.03 to 0.05 M with only about 50% hypochromicity observed. Furthermore, this DNA displayed two steps

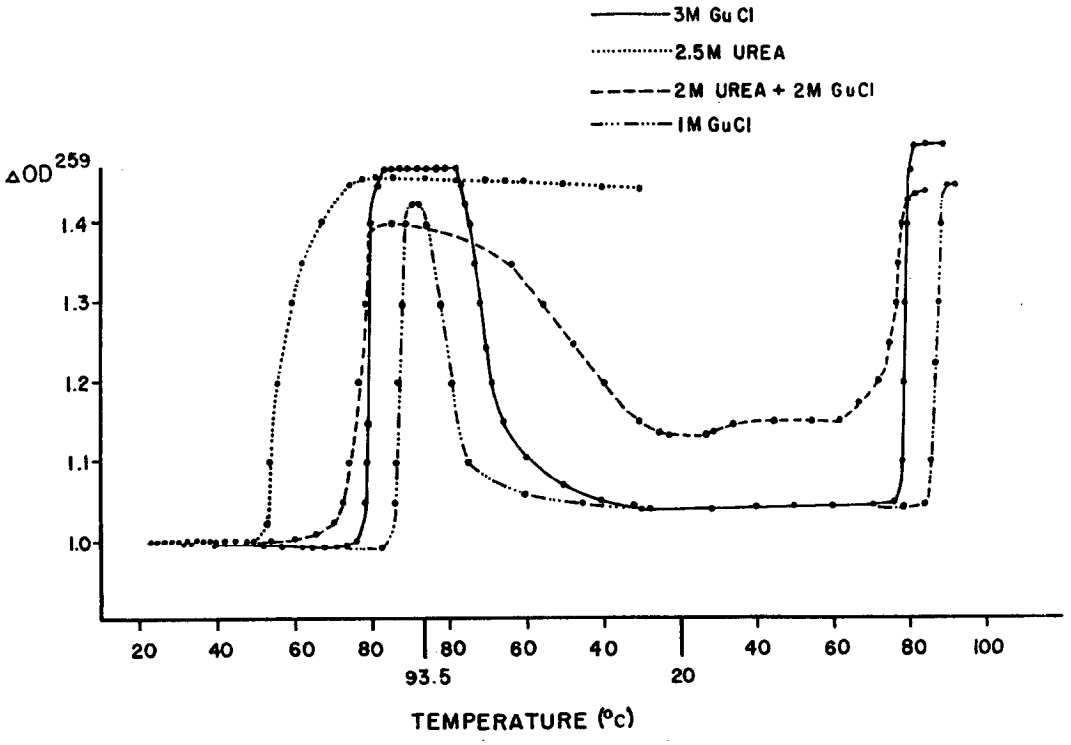

Fig. 2 - Thermal denaturation, renaturation and subsequent denaturation of DNA in solvents containing GuCl and/or urea. Temperature control was programmed at $\Delta T^{o}c$ = 1/min, changing directions when a maximal hyperchromicity or hypochromicity was reached. ΔOD^{259} was computed taking hysteresis into consideration.

of melting upon heating. The first melting experienced a broad transition breadth and a lower Tm, while the second step is identical to that of native DNA, suggesting an imperfect and partial renaturation. The presence of GuCl at 0.1 M allows better than 90% renaturation. Even with 8 M GuCl, an 80% hypochromic recovery was observed. This is in contrast to the observation that there is no renaturation of DNA in the presence of urea alone. That the presence of GuCl overcomes the effect of urea is also apparent in Figure 2.

The stability of a Watson-Crick DNA double helix (1953) is determined not only be electrostatic and hydrophobic interactions between the two polynucleotide chains, but also by the forces due to base stacking. At neutral pH, a DNA molecule is an anionic polyelectrolyte, having a single negative charge for each phosphate moiety. Indeed, it has been found that the Debye-Hückel screening radius of charged phosphate varies inversely with the square root of the ionic strength. At low ionic strengths, the charge repulsion of double helical DNA becomes a significant factor (MacGillivray and McMullen 1966). This is explained by the fact that during unwinding of a DNA, decreases in charge densities accompany increases in the exposure of hydrophobic bases to the solvent. By pertubating their hydrophobic bonds, a change in the solvent properties would further affect the stability of a DNA double helix (Gordon, Curnutte and Lark 1965, Sinanoglu and Abdulmur 1965).

Based on the results presented here, it is clear that GuCl functions at two different sites. At low ionic strength it stabilizes the DNA helical structure, as higher temperatures are required to denature the DNA with increasing GuCl. It is possible that the guanidinium ions here neutralize the charges of the phosphates of DNA. As a result, increasing concentrations of GuCl cause increasing charge repulsion between the two strands of a helical DNA leading to an eventual cooperative unwinding of the helix. This is reflected by the changes in transition breadth versus ionic concentration. At high concentrations of guanidinium ion, a disruption of the ordered helical structure is apparently involved. The dynamics of balancing its stabilization

effect is upset. Thus, with increasing ionic strength of GuCl, there is a lowering of Tm.

While mutual repulsion of the strands in a double helix accounts for denaturation, poor hydration of positively charged adenyl guanyl and cytidyl groups in the relatively non-polar core of the native molecule may also play a part. It should be pointed out that the solubility of guanosine is increased by GuCl, although changes with adenosine are not obvious (unpublished data). Changes in solubility may indeed imply an alteration in hydrophobic bonding (Levine, Gordon and Jencks 1962).

Our preliminary studies have shown that GuCl and urea do not exert an additive effect in Tm depression, neither are the effect of GuCl and NaCl accumulative. This indicates that GuCl action is different, at least in part, from both urea and salt, although some degree of competitiveness cannot yet be ruled out. The GuCl effect is similar with DNAs isolated from crab poly dAT, chick embryos and Hemophilus influenzae.

The present finding is in some way reminiscent of that of metal ion effects on DNA. The ions Zn^{++}, Cd^{++}, and Cu^{++}, interact with DNA via two major classes of electron donor sites, namely: the phosphate moieties of the deoxyribose phosphate backbone and the electron donor groups on the bases (Shin and Eichhorn 1968). The site of attack depends on the ionic strength and specific to the ion/DNA ratio (Eichhorn and Shin 1968). At a ion/DNA-phosphate less than unity, the phosphate groups are supposedly neutralized. In this study, guanidinium ions are present in excess amounts compared to the DNA-phosphate, but a dual effect is nevertheless realized.

Experimental and Methods

1. DNA preparation: T4 DNA was prepared from T4 $r^{+}h^{+}$ infected in E. coli B. Infection was made at a multiplicity of 0.1. Complete lysis was enhanced with lysozyme (Worthington) and chloroform, and the host nucleic acids hydrolyzed by pancreatic DNase and RNase (both Calbiochem). Phage was harvested by repeated centrifugation and DNA extracted with phenol following the procedure of

Thomas and Abelson (1967). All DNA solutions were concentrated to greater than 500 μg/ml and stored in SSC (0.15 M NaCl-0.015 M Na citrate pH 6.8) under refrigeration.

2. Reagents: Ultra pure GuCl (Mann Biochemical) and urea (Mallinckrodt) were used without further purification as they did not absorb UV. Other products of GuCl required purification on an activated charcoal column. Water was glass distilled (Kontes).

3. Methods: Absorbance changes at 259 mμ of DNA solutions were followed by a Beckman DU spectrophotometer coupled to a Gilford 2000 recorder. The temperature of the cell compartment was regulated by a Tamson constant-temperature circulator equipped with a Neslab temperature programmer. All spectrophotometric measurements were made using quartz cuvettes (Pyrocell) of 1.0 cm light path, 3 ml working capacity, with ground glass stoppers. DNA solutions were freshly diluted with the appropriate solvents and vacuum degassed before use. By increasing the temperature by 1.0°C/minute, the change in hyperchromicity was followed. The cooling rate was also 1.0°C/minute. In all measurements, DNA was adjusted to 30 μg/ml and 0.01 x SSC. Higher concentrations (two to four-fold)of DNA did not affect the results.

Acknowledgments

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